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学 位 の 種 類	博士（創薬科学）
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学位論文要旨

Abstract

In this study, to obtain potential photosensitizing anticancer agents with improved hydrophilicity and high tumor accumulation, a porphyrin framework, Pt(II)-based chemotherapeutic drug, and metal ions were combined (Ga-4cisPtTPyP, Ga-4transPtTPyP, In-4cisPtTPyP, In-4transPtTPyP Zn-4cisPtTPyP, Zn-4transPtTPyP, and 4Pt(dach)ClTPyP), and their photophysical and photochemical properties were investigated. All of these mixed-metal porphyrin complexes displayed reasonable water solubility, lack of aggregation, high singlet oxygen quantum yield due to Pt groups, and excellent photocytotoxicity. The impact of the presence of heavy metals, Zn(II), In(III), and Ga(III), on the photophysical and photochemical properties as well as PDT efficacy were studied. In addition, with accumulation in tumor tissue, 4Pt(dach)ClTPyP completely killed tumor cells, not simply displaying inhibition of tumor growth. The reasons for cell death may be attributed to high singlet oxygen quantum, internalization into nucleus, and an apoptosis pathway. Based on these experimental results, the peripheral combination of platinum(II) groups with a porphyrin framework, generating considerable $\Phi_{\Delta,}$ makes a substantial contribution to the anticancer activity in photodynamic therapy.

Introduction and Results

Photodynamic therapy (PDT), combining three components: light, singlet oxygen ($^1O_2$), and a photosensitizer, is a curative treatment widely used for the treatment of various forms of cancer.

Irradiated with light of wavelengths in the range 600–800 nm, the therapy determines singlet oxygen generation, which is an actively cytotoxic agent in PDT. Porphyrin-based sensitizers are thermodynamically and kinetically stable and show great retention or accumulation in tumors as the result of preferential binding to low-density lipoproteins. Functionalization with charged peripheral groups or insertion of metals into the core could make them water-soluble, markedly affecting their biodistribution and in vivo efficacy. For successful PDT treatment, an efficient photosensitizer must meet several conditions: (1) no cytotoxicity under dark, (2) reasonable cytotoxic $^1O_2$ generation ability under light, (3) a high absorption coefficient in the long wavelength region, (4) reasonable water-solubility, and (5) selective accumulation to the tumor tissue.

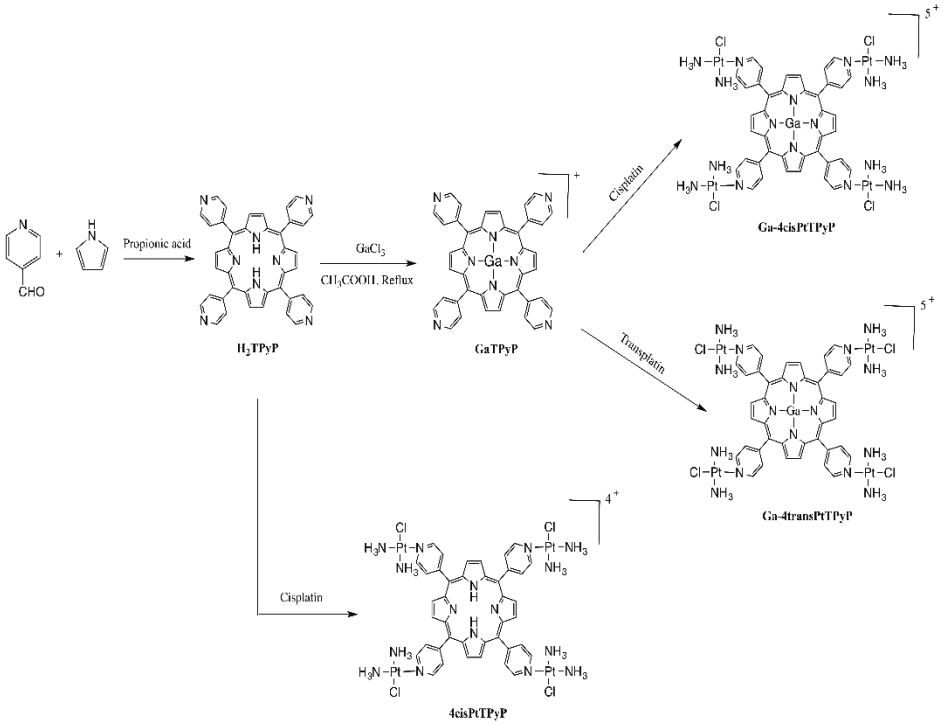
However, the notorious insolubility and aggregation of porphyrins in aqueous solutions negatively affect their cellular uptake and restrict in vivo activity and applications. Given these limitations, efforts have been devoted for developing water-soluble cationic porphyrins that can eliminate the formation of aggregates by increasing the electrostatic repulsion among charged functional groups. Meanwhile, some porphyrin and platinum conjugates have been synthesized with the purpose of improving both hydrophilicity and antitumor activity; these complexes exhibited excellent efficacy in sequential PDT treatments. Consequently, the system combining porphyrins and Pt-based complexes generated interesting synergistic treatment effects, achieving a tremendous enhancement in anticancer effects.

In light of the potential advantages, we have focused on porphyrins and metalloporphyrins substituted with platinum-based anticancer drugs, aiming to enhance their hydrophilicity, thereby

enabling in vivo evaluation. We also anticipated tumor-targeted effects. In this study, a porphyrin or metalloporphyrin framework and platinum(II)-based groups were combined to produce some new cationic porphyrins. Their photophysical and photochemical properties were investigated, and the photodynamic activities of these complexes in vitro and in vivo were evaluated. The complexes were found to manifest highly potent anticancer activity in vitro and in vivo.

Chapter 1 Water-soluble metalloporphyrinates with excellent photo-induced anticancer activity resulting from high tumor accumulation

To develop a water-soluble and tumor-targeted photosensitizer for PDT, a porphyrin framework containing the metal ion gallium(III) was combined with platinum(II)-based groups to produce two new pentacationic metalloporphyrinates (Ga-4cisPtTPyP and Ga-4transPtTPyP, Scheme 1). The mixed-metal porphyrinate, Ga-4cisPtPyP, is an efficient singlet oxygen generator because of heavy atom effect, more acidic p*K*a, and localization in cytosol. It also showed negative dark cytotoxicity caused by more hydrophilicity and slower and lower cellular uptake. In particular, Ga-4cisPtTPyP displayed a low IC_{50} value (Colon 26: 0.12 μM; Sarcoma 180: 0.08 μM, Table 1) under illumination and its phototoxic index up to 1,000. We proposed that the excellent photocytotoxicity, interaction with DNA, and apoptosis pathway could contribute to the anticancer activity of Ga-4cisPtPyP. With outstanding tumor accumulation (tumor/muscle ratio > 9), Ga-4cisPtTPyP almost completely inhibited tumor growth over two weeks in an in vivo PDT assay (Fig. 1). These results imply that Ga-4cisPtTPyP could be a promising anticancer agent for use in PDT.



Scheme 1. Synthesis of Ga-4cisPtTPyP, Ga-4transPtTPyP and 4cisPtTPyP.

Table 1 IC_{50} values (μM) toward Colon 26 and Sarcoma 180.

Compound	Colon 26[a]		PI[b]	Sarcoma 180[a]		PI[b]
	In dark	With light		In dark	With light	
Ga-4cisPtTPyP	>100	0.12±0.02	>833	>100	0.08±0.05	>1250
Ga-4transPtTPyP	22.68±8.60	0.14±0.02	162	68.47±8.87	0.16±0.02	428
4cisPtTPyP	10.55±1.39	0.14±0.01	75	2.23±0.78	0.17±0.08	13
GaTPyP	4.11±1.01	1.80±1.38	2.3	3.26±0.38	1.13±1.04	2.9
GaTPyP+4cisplatin	2.54±0.46	0.87±0.13	2.9	3.77±1.03	2.28±0.89	1.7
cisplatin	3.62±1.00	3.25±0.91	1.1	9.34±3.23	16.02±6.62	0.6

[a] Cancer cells were incubated for 24 h with different complexes.

[b] PI: phototoxic index

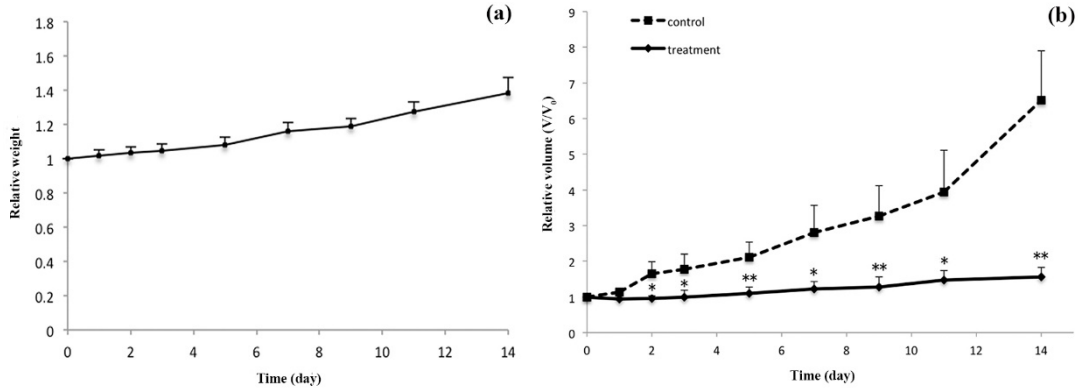


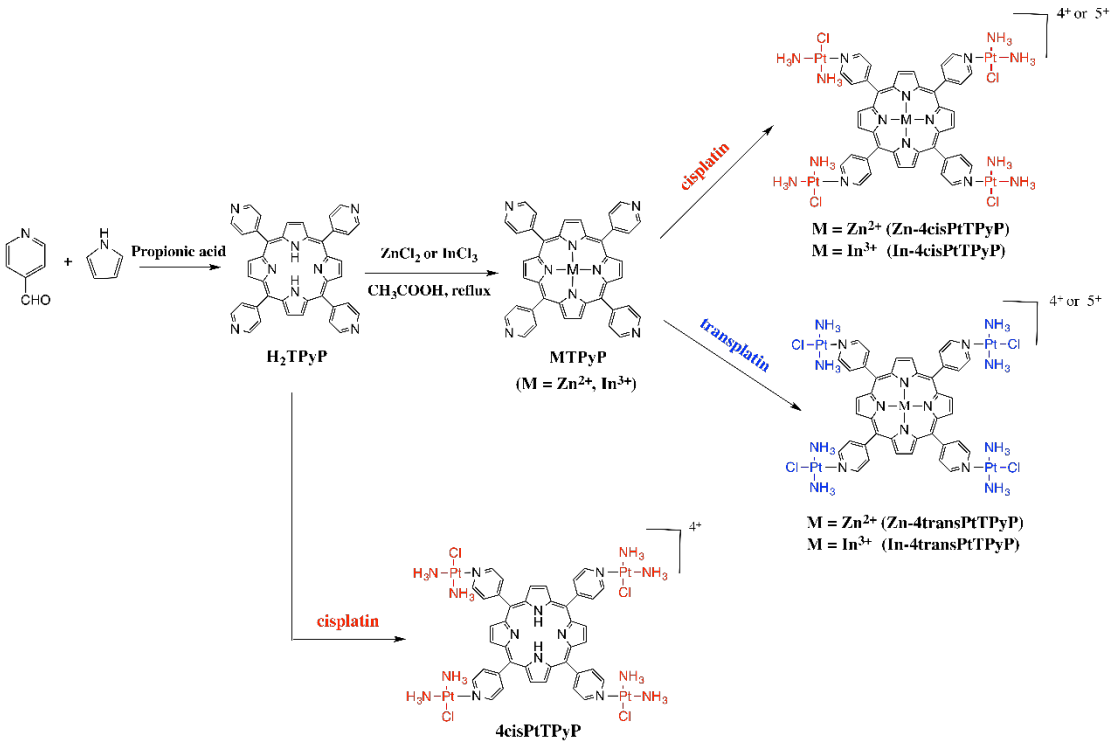
Fig. 1. (a) Relative weight after treatment of Ga-4cisPtTPyP on BALB/c mice; (b) Tumor growth curves of control group and Ga-4cisPtTPyP treated group. The tumor volumes were normalized to their initial sizes. The error bars represent the standard deviations of 5 mice per group. *, $p < 0.05$; **, $p < 0.01$.

Chapter 2 Effect of metalation on water-soluble cationic metalloporphyrins in photodynamic therapy

To further investigate the effect of metalation on water-soluble cationic metalloporphyrins in photodynamic therapy, M-4PtTPyP (M=Zn and In), namely Zn-4cisPtTPyP, Zn-4transPtTPyP, In-4cisPtTPyP, and In-4transPtTPyP, were synthesized and studied for the first time (Scheme 2). We demonstrated the impact of the presence of heavy metals, Zn, In, and Ga, on the photophysical and photochemical properties as well as PDT efficacy. The ranking of overall charges for these complexes, is listed as follows:

In-4cisPtTPyP, In-4transPtTPyP, Ga-4cisPtTPyP, and Ga-4transPtTPyP (5^+) > Zn-4cisPtTPyP and Zn-4transPtTPyP (4^+) > InTPyP and GaTPyP (1^+) > ZnTPyP (0)

From the results, an increase in the overall charge results in a decrease in log*P* values



Scheme 2. Synthesis of Zn-4cisPtTPyP, Zn-4transPtTPyP, In-4cisPtTPyP, and In-4transPtTPyP.

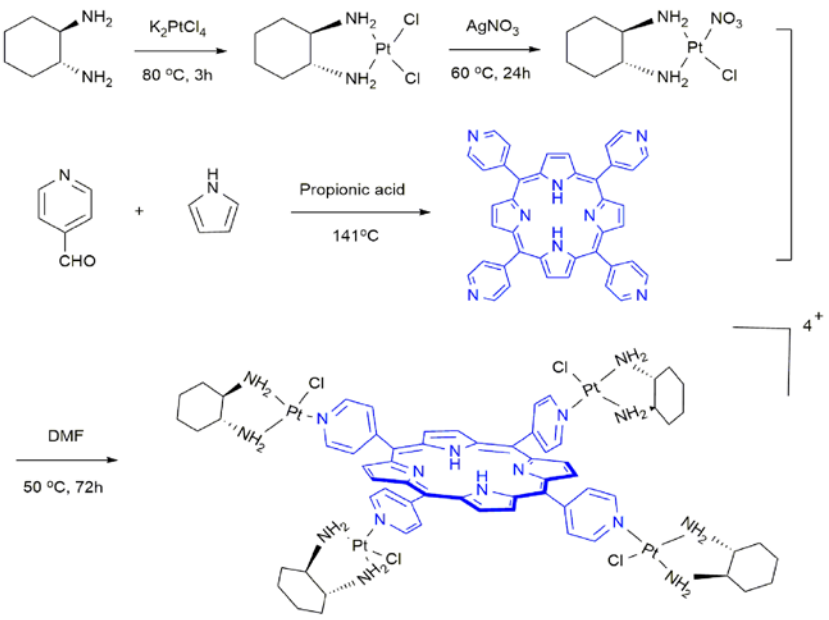
Table 2 The log*P* (*n*-octanol/water) values and quantum yields of singlet oxygen.

Compound	log*P*	Φ_Δ
Zn-4cisPtTPyP	-1.63±0.16	1.52±0.41
Zn-4transPtTPyP	-1.59±0.11	0.80±0.27
In-4cisPtTPyP	-1.97±0.19	0.76±0.07
In-4transPtTPyP	-1.92±0.26	0.62±0.28
Ga-4cisPtTPyP	-2.06±0.21	0.76±0.08
Ga-4transPtTPyP	-1.91±0.20	0.63±0.09
4cisPtTPyP	-1.77±0.08	0.57±0.03
ZnTPyP	1.19±0.07	0.35±0.07
InTPyP	0.96±0.06	0.35±0.10

and an increase in water solubility. These properties were affected significantly by changing the central metal cation in the studied platinum-porphyrin complexes. Additionally, the increasing of atom size of central metal enhanced the efficiency of singlet oxygen generation (Table 2). The presence of the heavy metal in porphyrins can decrease the energy difference between the singlet state and the excited triplet state of photosensitizers and increase its triplet state lifetime, in a process known as the heavy-atom effect. Although Zn-Pt porphyrins can generate the largest singlet oxygen and show stable under irridiation, these two complexes do not have the potential to serve for PDT since their poor solubility and low phototoxic indexes in vitro PDT assay. However, In-Pt porphyrins showed good photophysical and photochemical properties and it can be good candidates as Type II photosensitizers in photodynamic therapy applications.

Chapter 3 A platinum-porphyrin conjugate: an excellent cancer killer for photodynamic therapy

A cationic porphyrin of structural dependence on Pt moiety was further studied to develop another potential photosensitizing agent with high antitumor effect. In this part, a porphyrin framework as a carrier to tumor tissue and a Pt (II)-based chemotherapeutic drug were combined and characterized (Scheme 3). The positive charge-rich regions of this complex prohibit formation of aggregates by electrostatic repulsion and facilitate metal-to-ligand charge transition to increase singlet oxygen quantum. The tetracationic porphyrin-platinum(II) conjugate (4Pt(dach)ClTPyP) displayed reasonable water solubility, lack of aggregation, high singlet oxygen quantum yield, and excellent photocytotoxicity. This complex triggered caspase-3 induced apoptosis under irradiation. With accumulation in tumor tissue, 4Pt(dach)ClTPyP completely killed tumor cells, not simply displaying inhibition of tumor growth (Fig. 2). The reasons for cell death may be attributed to high singlet oxygen quantum, internalization into nucleus, and an apoptosis pathway. Based on these experimental results, 4Pt(dach)ClTPyP is a highly promising anticancer agent for PDT.



Scheme 3 Synthesis of 4Pt(dach)ClTPyP.

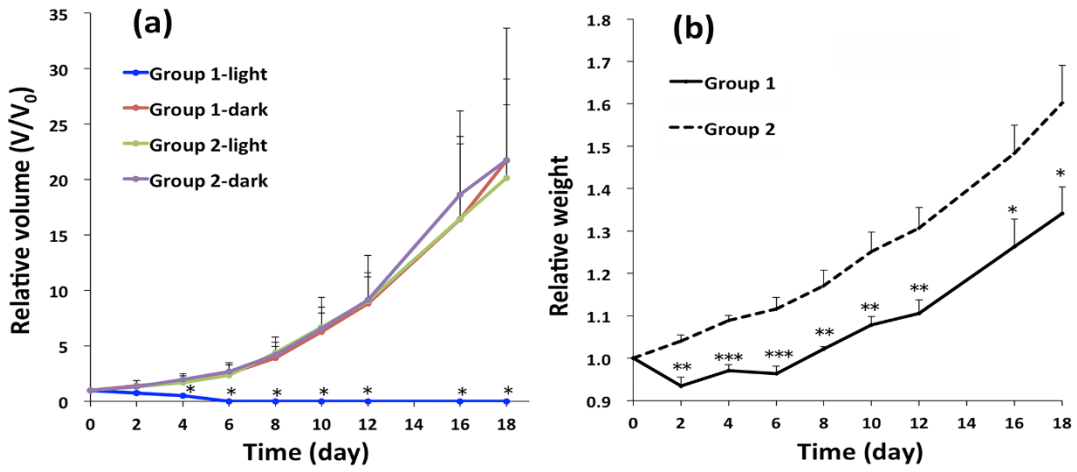


Fig. 2. In vivo PDT evaluation of 4Pt(dach)ClTPyP in Conlon26 tumor-bearing mice. (a) Tumor growth curves of group 1 (4Pt(dach)ClTPyP, n = 4) and group 2 (Control, n = 4). The tumor volumes were normalized to their initial sizes. (b) Relative weight of group 1 and group 2. The error bars represent the standard deviations of 4 mice per group. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$.

審査結果の要旨

PDT(photodynamic therapy：光線力学療法)は光照射によって光感受性薬剤から一重項酸素を発生させて標的を攻撃する手法で，近年肺がん治療等に応用されているが，よりよいPDT薬剤が望まれている。本研究は，テトラピリジルポルフィリン(TPyP)に複数金属を異なる部位へ導入し，１）高い光活性，２）腫瘍集積性などの動態優位性，３）高いin vitroおよびin vivo抗がん活性，を発現させることを目的とした。光照射による一重項酸素発生能は，混合金属系M-4Pt-TPyPが最も高く，次いで側鎖白金錯体4Pt-TPyPであった。一重項酸素発生能と低いIC50値（高い活性）は比例しており，Pt部位の重要性が示された。混合金属系においては高い腫瘍集積性，光非照射下の低毒性等，動態での優位性が認められた。マウス動態からは標的臓器内での錯体の分解が示され，抗がん活性への寄与が示唆された。担がんマウスを用いたPDT治療実験においては非常に優れた治療効果を認め，よりよいPDT薬剤の開発に際してポルフィリンの混合金属錯体化が有用な方法であることを明らかにした。これらの知見は創薬の学術面で大きく貢献している。以上より，本論文は博士（創薬科学）に値すると判断される。